

IN THE SPECIFICATION

- (1) Delete the paragraphs on page 10, line 11 to page 11, line 8 and replace them with:

α Chain Thr 48:	DSDVYITDKTVLDMRSMDFK (amino acids 39-58 of exon 1 of the TRAC*01 gene; SEQ ID NO:2)
α Chain Thr 45:	QSKDSDVYITDKTVLDMRSM (amino acids 36-55 of exon 1 of the TRAC*01 gene; SEQ ID NO:3)
α Chain Tyr 10:	DIQNPDPAVYQLRDSKSSDK (amino acids 1-20 of exon 1 of the TRAC*01 gene; SEQ ID NO:4)
α Chain Ser 15:	DPAVYQLRDSKSSDKSVCLF (amino acids 6-25 of exon 1 of the TRAC*01 gene; SEQ ID NO:5)
β Chain Ser 57:	NGKEVHSGVSTDPQPLKEQP (amino acids 48-67 of exon 1 of the TRBC1*01 & TRBC2*01 genes; SEQ ID NO:6)
β Chain Ser 77:	ALNDSRYALSSRLRVSATFW (amino acids 68-87 of exon 1 of the TRBC1*01 & TRBC2*01 genes; SEQ ID NO:7)
β Chain Ser 17:	PPEVAVFEPSEAEISHTQKA (amino acids 8-27 of exon 1 of the TRBC1*01 & TRBC2*01 genes; SEQ ID NO:8)
β Chain Asp 59:	KEVHSGVSTDPQPLKEQPAL (amino acids 50-69 of exon 1 of the TRBC1*01 & TRBC2*01 genes; SEQ ID NO:9)
β Chain Glu 15:	VFPPEVAVFEPSEAEISHTQ (amino acids 6-25 of exon 1 of the TRBC1*01 & TRBC2*01 genes; SEQ ID NO:10)

- (2) Delete the paragraph on page 11, lines 10-17 and replace it with:

In other species, the TCR chains may not have a region which has 100% identity to the above motifs. However, those of skill in the art will be able to use the above motifs to identify the equivalent part of the TCR α or β chain and hence the residue to be mutated to cysteine. Alignment techniques may be used in this respect. For example, ClustalW, available on the European Bioinformatics Institute website (<http://www.ebi.ac.uk/index.html>) can be used to compare the motifs above to a

particular TCR chain sequence in order to locate the relevant part of the TCR sequence for mutation.

- (3) Delete the paragraph on page 11, line 30 to page 12, line 25 and replace it with:

Mouse C α soluble domain:

PYIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKTVLDMK
AMDSKSNAGIAWSNQTSTCQDIFKETNATYPSSDVP (SEQ ID NO:11)

Mouse C β soluble domain:

EDLRNVTTPKVSLFEPKAEIANKQKATLVCLARGFFPDHVELSWVWNGREV
HSGVSTDPQAYKESNYSYCLSSRLRVSAFWHNP RNHFRQVQFHGLSEEDK
WPEGSPKPVTONISAEAWGRAD (SEQ ID NO:12)

Murine equivalent of human α Chain Thr 48: ESGTFITDKTVLDMKAMDSK (SEQ ID NO:13)

Murine equivalent of human α Chain Thr 45: KTMESGTFITDKTVLDMKAM (SEQ ID NO:14)

Murine equivalent of human α Chain Tyr 10: YIQNPEPAVYQLKDPRSQDS (SEQ ID NO:15)

Murine equivalent of human α Chain Ser 15: AVYQLKDPRSQDSTLCLFTD (SEQ ID NO:16)

Murine equivalent of human β Chain Ser 57: NGREHVHSGVSTDPQAYKESN (SEQ ID NO:17)

Murine equivalent of human β Chain Ser 77: KESNYSYCLSSRLRVSAFW (SEQ ID NO:18)

Murine equivalent of human β Chain Ser 17: PPKVSLFEPKAEIANKQKA (SEQ ID NO:19)

Murine equivalent of human β Chain Asp 59: REVHSGVSTDPQAYKESNYS (SEQ ID NO:20)

Murine equivalent of human β Chain Glu 15: VTPPKVSLFEPKAEIANKQ (SEQ ID NO:21)

- (4) Delete the paragraphs on page 23, lines 5-15 and replace them with:

For mutating A6 Tax threonine 48 of exon 1 in TRAC*01 to cysteine, the following primers were designed (mutation shown in lower case):

5'-C ACA GAC AAA t**g**T GTG CTA GAC AT (SEQ ID NO:22)

5'-AT GTC TAG GAG Aca TTT GTC TGT G (SEQ ID NO:23)

For mutating A6 Tax serine 57 of exon 1 in both TRBC1*01 and TRBC2*01 to cysteine, the following primers were designed (mutation shown in lower case):

5'-C AGT GGG GTC t**g**C ACA GAC CC (SEQ ID NO:24)

5'-GG GTC TGT GCa GAC CCC ACT G (SEQ ID NO:25)

- (5) Delete the paragraphs on page 25, line 15 to page 26 line 8 and replace them with:

Briefly, the alpha and beta chains of the A6 dsTCR were amplified by PCR using primers containing restriction sites as shown in FIG. 4, i.e.:

Alpha 5' primer: ccaaggccatatgcagaaggaagtggagcagaactct (SEQ ID NO:26)

Alpha 3' primer: ttgggcccgccggatccgccccgggggaactttctgggctgggg (SEQ ID NO:27)

Beta 5' primer: tccccggggcgggatccgcgggcccaacgctggtgtcactcag (SEQ ID NO:28)

Beta 3' primer: ggggaagcttagtctgctctacccaggcctcg (SEQ ID NO:29)

The ~~two~~ ~~Two~~ fragments thus generated were PCR stitched using the 5' alpha and 3' beta primers to give a single-chain TCR with a short linker containing the sites XmaI-BamHI-ApaI. This fragment was cloned into pGMT7. The full length linker was then inserted in two stages, firstly a 42 bp fragment was inserted using the XmaI and BamHI sites:

5'-CC GGG GGT GGC TCT GGC GGT GGC GGT TCA GGC GGT GGC G-3' (SEQ ID NO:30)

3'-C CCA CCG AGA CCG CCA CCG CCA AGT CCG CCA CCG CCT AG-5' (SEQ ID NO:31)

Secondly, a 48 bp fragment was inserted using the BamHI and ApaI sites to create a 90 bp linker between the 3' end of the alpha chain and the 5' end of the beta chain. The 48 bp fragment was made by PCR extension of a mixture of the following oligos:

5'- GC GGA TCC GGC GGT GGC GGT TCG GGT GGC GGT GGC TC-3' (SEQ ID NO:32)

3'- CCA AGC CCA CCG CCA CCG AGT CCG CCA CCG CCC GGG TG -5' (SEQ ID NO:33)

**** Underlining in SEQ ID NO:32 is present in the original specification.****

- (6) Delete the paragraphs on page 21, line 14 to page 22, line 24 (description of the drawings).
- (7) Insert the following paragraphs on page 5, after line 13:

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1a and 1b show respectively the nucleic acid sequences of the α (SEQ ID NO:35) and β (SEQ ID NO:36) chains of a soluble A6 TCR, mutated so as to introduce a cysteine codon. The shading indicates the introduced cysteine codons.

FIG. 2a shows the A6 TCR α chain extracellular amino acid sequence (SEQ ID NO:37), including the T₄₈ → C mutation (underlined) used to produce the novel disulphide inter-chain bond, and FIG. 2b shows the A6 TCR β chain extracellular amino acid sequence (SEQ ID NO:38), including the S₅₇ → C mutation (underlined) used to produce the novel disulphide inter-chain bond.

FIG. 3 shows the DNA (SEQ ID NO:39) and amino acid (SEQ ID NO:34) sequences of the Gly/Ser linker (30mer).

FIG. 4 summarizes the cloning strategy used to produce the scDiS A6 TCR.

FIG. 5a shows the DNA sequence of the scDiS A6 TCR (SEQ ID NO:40).

FIG. 5b shows the amino acid sequence of the scDiS A6 TCR (SEQ ID NO:41).

FIG. 6 illustrates the elution of the scDiS A6 TCR protein from a POROS 50HQ ion exchange column using a 0-500 mM NaCl gradient, as indicated by the straight line.

FIG. 7 shows the results of both reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels of fractions A15, B10, B9 and B3 from the column run illustrated by FIG. 6. Fractions B9 and B10 clearly contain protein corresponding to the expected size of the scDiS A6 TCR.

FIG. 8 illustrates the elution of the scDiS A6 TCR elution from a Superdex 200 gel filtration column of fractions B10-B7 from the ion exchange column run shown in FIG. 6.

FIG. 9 shows the results of both reducing SDS-PAGE (Coomassie-stained) and non-reducing SDS-PAGE (Coomassie-stained) gels of fractions B8, B7, B3 and B2 from the gel filtration column run illustrated by FIG. 8. Fraction B7 clearly contains protein corresponding to the expected size of the scDiS A6 TCR.

FIG. 10 is a final gel filtration run into BIAcore buffer of the concentrated fractions B9-B6 of the gel filtration run shown in FIG. 8. The scDiS A6 TCR elutes as a single major peak.

FIG. 11. BIAcore data for binding of the scDiS A6 TCR to HLA-A2 TAX.